Docket No. 050992.0201.00USCP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Itzhak Bentwich
App. No.: 10/707,147
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Art Unit: 1635
Examiner: BOWMAN, AMY HUDSON
Title: BIOINFORMATICALLY DETECTABLE GROUP OF NOVEL REGULATORY GENES AND USES THEREOF

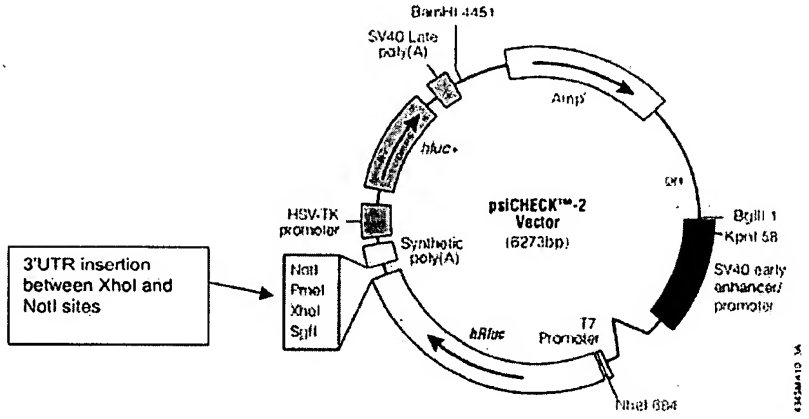
DECLARATION OF AYELET CHAJUT, PH.D.

Dear Sir:

I, Ayelet Chajut, Ph.D., hereby declare as follows:

1. I am the Executive Vice President, R&D at Rosetta Genomics, Ltd. ("Rosetta"). A true and correct copy of my Curriculum Vitae is attached to this declaration as Exhibit A.
2. I have 22 years of experience designing and performing experiments in the field of molecular biology, 2.5 of which were related to miRNA biology. I have also worked in the biotechnology industry for 10 years.
3. As a result of my work as Executive Vice President, R&D and experience in the field of molecular biology, I supervised and conducted the experiments described herein at items 4-6.
4. In order to confirm that the miRNA hsa-miR-196b affects mRNA levels of the target LHFPL2, a partial sequence from the 3'UTR of this target, which is predicted to contain a hsa-miR-196b binding site, was fused to the 3'end of a Renilla luciferase mRNA expression construct. Specifically, a 365 bp fragment

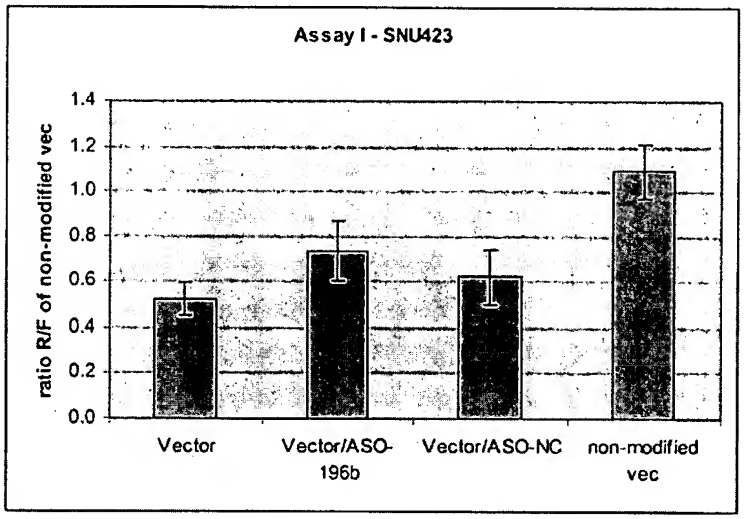
of the 3'UTR of LHFPL2 containing the miRNA binding site was cloned into the 3'UTR of Renilla luciferase in the psiCHECK-2 dual luciferase plasmid (GeneScript), as depicted below.



This reporter vector was introduced into Hep3B and SNU423 cells as follows. Cells of each line were plated at a density of 3000 and 4000 cells per well, respectively, on white collagen coated plates with a transparent bottom. Cells were transfected the next day with the reporter vector, and one of the following: an anti-sense oligonucleotide ("ASO") designed to specifically inhibit hsa-miR-196b, or a control ASO that specifically binds to let7b (negative control, or "NC") . Transfection was performed using 0.3μL of Lipofectamine 2000 per well (Invitrogen, Cat#11668027). Twenty-four hours after transfection, luminescence was assayed using the Dual Luciferase reporter assay kit (Promega, Cat#E1961), and measured using a Luminoskan Ascent (Thermo). Levels of firefly luciferase, which was produced from the same reporter vector as the Renilla luciferase/LHFPL2 3'UTR fusion, were used to normalize transfection efficiency. Additionally, a psiCHECK2 vector containing both Renilla and firefly luciferases, but lacking the LHFPL2 3'UTR fusion, was used as a reference for constitutive luciferase expression. The levels of Renilla luciferase were expressed as a ratio between the level measured from

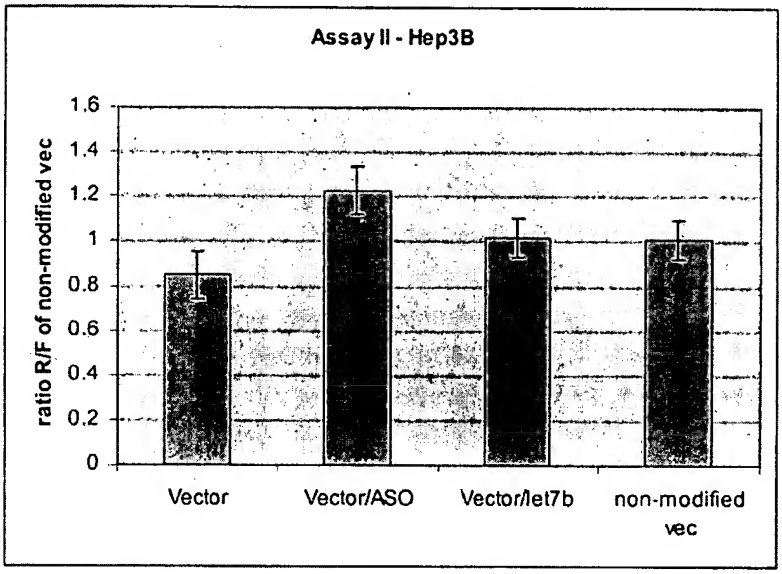
the various treatments and the level measured from cells transfected with the unaltered psiCHECK2 control vector. The assays were conducted twice, and the results are shown below.

5. The effects of the ASO to hsa-miR-196b on the Renilla luciferase/LHFPL2 3'UTR fusion as measured in the first assay are shown below.



In comparison to SNU423 cells transfected with the psiCHECK2 control vector with no LHFPL2 3'UTR (non-modified vector), cells transfected with the Renilla luciferase/LHFPL2 3'UTR fusion expressed lower levels of Renilla luciferase (first bar; "Vector"). Specifically, levels in SNU423 cells decreased from about 1.1 to 0.50. Compared to luciferase levels produced by Renilla luciferase/LHFPL2 3'UTR alone, adding the hsa-miR-196b ASO resulted in an increase from about 0.50 to nearly 0.75 in SNU423 cells (second bar; "Vector/ASO-196b"). SNU423 cells transfected with the Renilla luciferase/LHFPL2 3'UTR construct and the let7B ASO (third bar; "Vector/ASO-NC") exhibited luciferase levels of 0.63.

6. The effects of the ASO to hsa-miR-196b on the Renilla luciferase/LHFPL2 3'UTR fusion as measured in the second assay are shown below.



In comparison to Hep3B cells transfected with the psiCHECK2 control vector with no LHFPL2 3'UTR (non-modified vector) (last bar;), cells transfected with the Renilla luciferase/LHFPL2 3'UTR fusion expressed lower levels of Renilla luciferase (first bar; "Vector"). Specifically, levels in Hep3B cells decreased from about 1.00 to 0.85. Compared to luciferase levels produced by Renilla luciferase/LHFPL2 3'UTR alone, adding the hsa-miR-196b ASO resulted in an increase from about 0.85 to nearly 1.25 in Hep3B cells (second bar; "Vector/ASO"). Hep3B cells transfected with the Renilla luciferase/LHFPL2 3'UTR construct and the let7B ASO (third bar; "Vector/let7b") exhibited luciferase levels of about 1. .

7. I solemnly declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or

both, under 35 U.S.C. § 1001, and may jeopardize the validity of the application or any patent issuing thereon.

Dated: 27 Nov. 2008 By: A. Chajut
Ayelet Chajut, Ph.D.

Exhibit A

CURRICULUM VITAE

AYELET CHAJUT

PERSONAL

Name: Ayelet Chajut
Date of Birth: 5th August, 1962
Place of Birth: Israel
Family Status: Married + 2
Military Service: 1980-1982
Phone: 03-5401981, 052-4287229
e. mail address: mailto:ayelet_ch@rosettagenomics.com

PROFESSIONAL EXPERIENCE

2007- Executive Vice President R&D, Head Molecular Biology, at Rosetta-Genomics.

2006- 2007 Vice President Therapeutics, at Rosetta-Genomics.
In this capacity, I am responsible for the development of new drugs based on microRNAs.

2005-2006 Director of Science & Technology at Quantomix, Ltd.
In this capacity I am responsible for development of biological applications of the WETSEM technology, mainly in the field of metabolic disorders focusing on the drug development and diagnostic areas, collaborations with academia and pharmaceutical companies.

2003-2005 Vice President Research, at Quark Biotech, Inc.
In this capacity, in addition to my previous tasks, I was responsible to the drug discovery units including: Protein expression and purification, bioassay development. Chemical screening, data analysis, hits selection and validation.

2002-2003 Senior director of Target Discovery and Validation, at Quark Biotech, Inc
In this capacity, in addition to my previous tasks, I was responsible to target gene validation processes in 5 different pathology-related research teams

2000-2002 Director of Target Discovery, at Quark Biotech, Inc
In this capacity I headed the multidisciplinary candidate genes selection committee responsible for nominating and selecting the genes that QBI

should focus research and development efforts on. Responsible for Gene-discovery process units (RNA, cDNA libraries, microarray printing, bioinformatic and data analysis).

1998-2000 Senior scientist, in charge of "Stem Cells" research at Quark Biotech. In this capacity I designed a robust gene discovery program aimed at elucidating the mechanisms of pluripotency of Embryonic & Hematopoietic stem cells and identification of new targets. I was responsible for carrying out these plans by managing the internal research efforts as well as collaborations with several leading researchers in the field.

1989-1994 Laboratory instructor and tutor of 3rd year medical students, Department of Microbiology, Faculty of Medicine, Tel-Aviv University

1993-1999 Managing the "Virology" course in the Open University of Israel, Both from the academic aspect and the administrative aspect.

1997-1998 Project manager, Orit – technological R&D center Ltd, Ariel, Israel.

EDUCATION:

1994-1997 Post Doctoral studies in the Laboratory of Prof. Sara Lavi, Department of Cell Research and Immunology, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv. Main study: Molecular and biochemical characterization of protein phosphatase 2C (PP2C) in eukaryotic cells; Identification of a putative new cell cycle regulator.

1989-1994 Studies towards Ph.D. degree in the Laboratory of Prof. Abraham Yaniv and Prof. Arnona Gazit in the Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv. Thesis: "Lymphoproliferative disease virus of turkeys Studies of oncogenetic mechanism".

1988-1989 Studies in the Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv. M.Sc. degree ***(summa cum laude)***.

1983-1986 Studies in the Faculty of Agriculture, Hebrew University, Jerusalem. B.Sc. degree ***(cum laude)***.

RESEARCH EXPERIENCE

Molecular biology, Protein analysis, Cell culture, in vivo models, Bioinformatics, Microarray design and result analysis,. Bioassay development, HTS screening.

Publications

Articles:

1. Gak, E., Yaniv, A., Chajut, A., Ianconescu, M., Tronick, S.R. and Gazit, A. 1989. *Molecular cloning of an oncogenic replication - competent virus that causes lymphoproliferative disease in turkeys.* J. Virol. 63: 2877 - 2880.

2. Chajut, A., Yaniv, A., Avivi, L., Bar-am, I., Tronick, S.R. and Gazit, A. 1990. *A novel approach for establishing common or random integration loci for retroviral genomes.* Nucleic Acid Res. 15: 4299.

3. Chajut, A., Sarid, R., Gak, E., Yaniv, A., Garry, Tronick, S.R. and Gazit, A. 1992. *The lymphoproliferative disease virus of turkeys is a representative of a distinct class within the retroviridae, evolutionary related to the avian sarcoma- leukemia viruses.* Gene 122: 349 - 354.

4. Sarid, R., Chajut, A., Malkinson, M., Tronick, S.R., Gazit, A. and Yaniv, A. 1994. *Diagnostic test for lymphoproliferative disease virus of turkeys, using the polymerase chain reaction.* Am. J. Vet. Res. 55: 769 - 772.

5. Sarid, R., Chajut, A., Gak, E., Oroszlan, S., Tronick, S.R., Yaniv, A. and Gazit, A. 1994. *Nucleotide sequence and genome organization of a biologically active provirus of the lymphoproliferative disease virus of turkeys.* Virology 204: 648 - 691.

6. Yaniv, A., Sarid, R., Chajut, A., Gak, E., Altstock, R., Tronick, S.R. and Gazit, A. 1995. *The lymphoproliferative disease virus (LPDV) of turkeys.* Isr. J. Veter. Med. 50: 87-95.

7. Chajut, A., Gazit, A. and Yaniv, A. 1996. *The turkey c-rap1A proto-oncogene is expressed via two distinct promoters.* Gene 177: 7-10.

8. Seroussi E, Shani N, Ben-Meir D, Chajut A, Divinski I, Faier S, Gery S, Karby S, Kariv-Inbal Z, Sella O, Smorodinsky NI and Lavi S. 2001. *Uniquely conserved non-translated regions are involved in generation of the two major transcripts of protein phosphatase 2Cbeta.* J Mol Biol. 312:439-51.

9. Shoshani T, Faerman A, Mett I, Zelin E, Tenne T, Gorodin S, Moshel Y, Elbaz S, Budanov A, Chajut A, Kalinski H, Kamer I, Rozen A, Mor O, Keshet E, Leshkowitz D, Einat P, Skaliter R. and Feinstein E. 2002. *Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis.* Mol Cell Biol. 22:: 2283-93.

10. Budanov AV, Shoshani T, Faerman A, Zelin E, Kamer I, Kalinski H, Gorodin S, Fishman A, Chajut A, Einat P, Skaliter R, Gudkov AV, Chumakov PM and Feinstein E. 2002. *Identification of a novel stress-responsive gene Hi95 involved in regulation of cell viability.* Oncogene. 21: 6017-31.

11. Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, Benjamin H, Shabes N, Tabak S, Levy A, Lebanony D, Goren Y, Silberschein E, Targan N, Ben-Ari A, Gilad S, Sion-Vardy N, Tobar A, Feinmesser M, Kharenko O, Nativ O, Nass D, Perelman M, Yosepovich A, Shalmon B, Polak-Charcon S, Fridman E, Avniel A, Bentwich I, Bentwich Z, Cohen D, Chajut A, Barshack I. 2008. *MicroRNAs accurately identify cancer tissue origin.* Nat Biotechnol. 26:462-9.

12. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y and Chajut A. 2008. *Serum microRNAs are promising novel biomarkers.* PLoS ONE. 5:e3148.

Patents:

1. Chajut, A. 20032. Methods of using colony stimulating factors in the treatment of tissue damage and ischemia.
Patent N. US 20020198150

2. Chajut, A., Levinson M. and Skaliter R. 2003. 76A11 polypeptide and uses thereof. Patent N. US 20030157111

3. Byk T. and Chajut, A. 2004. Human protein sFRP1 and therapeutic use for induction of stem cell proliferation. Patent N. US 2004265995.

4. Byk T. Chajut, A. and Visser J 2004. Ctla-2 and uses thereof in the induction of stem cells. Patent N. US 200411340

Chapters in books:

1. Yaniv, A., Sarid, R., Chajut, A., Gak, E., Altstock, R., Smythers, G.W., Tronick, S.R. and Gazit, A. 1992. The lymphoproliferative disease virus (LPDV) of turkeys: an acute retrovirus lacking an oncogene. p. 163-175. In: Frensdorff, A. (ed.), Frontiers in cancer research. "Ramot" Publ. Tel-Aviv University.